Claims

1. An immunoassay for human brain natriuretic peptide, hBNP, comprising the steps of:

(a) contacting a solution suspected of containing hBNP with an enzyme-conjugated or radioisotope-labeled Fab' fragment of an antibody which is reactive with a first, N-terminal region of hBNP and an antibody reactive with a second, C-terminal region of hBNP having an amino acid sequence lys-val-leu-arg-arg-his (SEQ ID NO:2), to produce complexes of said enzyme-conjugated or radioisotope-labeled Fab' fragment, said hBNP, and said antibody reactive with a second region of hBNP;

(b) contacting said complexes of step (a) with an immobilized antibody reactive with the Fc fragment of said antibody reactive with a second region of hBNP to produce further complexes of said enzyme-conjugated or radioisotope-labeled Fab' fragment, said hBNP, said antibody reactive with a second region of hBNP, and said immobilized antibody;

(c) recovering and washing said further complexes of step (b);

(d) when an enzyme-conjugated antibody is employed in step (a), contacting said further complexes of step (c) with a substrate of said enzyme in an appropriate reaction buffer and

incubating so as to allow formation of the enzymatic reaction end product;

(e) determining the amount of said end product formed in step (d) when an enzyme-conjugated antibody is employed in step (a), or determining the amount of radioactivity bound to said further complexes of step (c) when a radioisotope-labeled antibody is employed in step (a); and

(f) relating the amount of said end product formed in step (e) or the amount of radioactivity bound to said further complexes of step (e) to the amount of said hBNP via the use of a standard curve for hBNP.

2. The immunoassay of claim 1, wherein said Fab' fragment of an antibody which is reactive with a first region of hBNP recognizes the intramolecular disulfide bridged loop structure of hBNP.

3. The immunoassay of claim 2, wherein said first antibody is produced by hybridoma KY-hBNP-II, FERM BP-2863.

4. The immunoassay of claim 1, herein said second antibody is produced by hybridoma BC203, FERM BP-3515.

5. The immunoassay of claim 4, wherein said first antibody recognizes the intramolecular disulfide bridged loop of hBNP.

6. An immunoassay for human brain natriuretic peptide, hBNP, comprising the steps of:

(a) contacting a solution suspected of containing hBNP with an enzyme-conjugated or radioisotope-labeled Fab' fragment of an antibody which is reactive with a first, N-terminal region of hBNP and an antibody that specifically binds to a second, C-terminal region of hBNP, having an amino acid sequence lys-val-leu-arg-arg-his (SEQ ID NO:2), which binding site includes the C-terminal his residue, to produce complexes of said enzyme-conjugated or radioisotope-labeled Fab' fragment, said hBNP, and said antibody reactive with a second region of hBNP;

(b) contacting said complexes of step (a) with an immobilized antibody reactive with the Fc fragment of said antibody reactive with a second region of hBNP to produce further complexes of said enzyme-conjugated or radioisotope-labeled Fab' fragment, said hBNP, said antibody reactive with a second region of hBNP, and said immobilized antibody;

(c) recovering and washing said further complexes of step (b);

(d) when an enzyme-conjugated antibody is employed in step (a), contacting said further complexes of step (c) with a substrate of said enzyme in an appropriate reaction buffer and incubating so as to allow formation of the enzymatic reaction end product;

(e) determining the amount of said end product formed in step (d) when an enzyme-conjugated antibody is employed in step (a), or determining the amount of radioactivity bound to said further complexes of step (c) when a radioisotope-labeled antibody is employed in step (a); and

(f) relating the amount of said end product formed in step (e) or the amount of radioactivity bound to said further complexes of step (e) to the amount of said hBNP by a standard curve for hBNP.

7. The immunoassay of claim 4, wherein said first antibody is produce by hybridoma KY-hBNP, FERM BP-2863.

8. An immunoassay for human brain natriuretic peptide, hBNP, comprising the steps of:

(a) contacting a solution suspected of containing hBNP with a Fab' fragment of an antibody which is reactive with a first, N-terminal region of hBNP and an antibody that specifically binds to a second, C-terminal region of hBNP, having an amino acid sequence lys-val-leu-arg-arg-his (SEQ ID

NO:2), which binding site includes the C-terminal his residue, to produce complexes of said Fab' fragment, said hBNP, and said antibody reactive with a second region of hBNP;

(b) contacting said complexes of step (a) with an immobilized antibody reactive with the Fc fragment of said antibody reactive with a second region of hBNP to produce further complexes of said Fab' fragment, said hBNP, said antibody reactive with a second region of hBNP, and said immobilized antibody;

(c) recovering and washing said further complexes of step (b);

(d) determining the amount of said further complexes and

(e) relating the amount of said further complexes determined in step (d) to the amount of said hBNP by a standard curve for hBNP.